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EFFECT OF GnRH INJECTION DURING SUMMER ON STEROID HORMONES AND LIPIDOGRAM PROFILES IN MALE FARM ANIMALS

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SUMMARY

The present study aimed to investigate the effect of a synthetic GnRH (Fertagyl®) injection during summer on steroid hormones levels and lipidogram in male farm animals.

Six healthy mature males farm animals (from each of cattle, buffalo, sheep and goats) were divided into 2 comparable equal groups. The first group was kept as a control, while the second group was injected (i.m) with 250 and 100μg GnRH for large and small animals, respectively. Blood samples were collected just before (0 time), then every an hour for 6 times post injection. Plasma samples were assayed for testosterone, estradiol 17β and cortisol levels. Also, concentrations of total lipids, cholesterol and triglycerides were determined.

Results of the present investigation reported that GnRH injection induced marked increases in testosterone (for 3-4 hrs.), decreases in estradiol-17β (except in buffaloes) and little changes in cortisol levels in male farm animals during summer. Results of lipidogram revealed non significant changes, however, total lipids and triglycerides values tended to increase and cholesterol tended to be decreased with the advance of time following GnRH injection in all studied males. Moreover, correlation coefficients were recorded among the studied parameters.

It is concluded that treatment of male farm animals with GnRH during summer may increase sexual desire and fertility.

INTRODUCTION

Sires significantly contribute to herd fertility, since high incidence of infertility problems in farm animals had been attributed to breeding with low fertile males (Vale, 1997). In the same time, reduced lipido, high incidence of sperm abnormalities and testicular degeneration especially during hot months of the year are the major causes of male infertility in buffaloes (Ahmed and Nada, 1994).

Male fertility could be improved through application of exercise, doushing, sanitary housing and false mounting (Dominguez et al., 1994), nutritional supplementation (Brown, 1994; El-Sheshtawy et al., 1998) and hormonal interference (Mehta et al., 1992).

Among hormonal preparations used nowadays, GnRH and its synthetic analogues could be used efficiently for improving fertility of both female (Ullah et al., 1996) and male(Anderson,1992) farm animals. Injection of GnRH caused a predictable release of LH in cow-bulls (El-Azab et al., 1997), buffalo bulls (Mehta et al., 1992) and rams (Xu et al., 1991) .It leads to significant improvement in libido ,ejaculate volume, sperm number and fructose content of semen (El Azab et al.,1997).

Summer heat stress impairs reproductive performance in farm animals. The primary reproductive responses to heat stress include reduced intensity of estrus in females and low sex drive in males with consequent low fertility (Ullah et al.,1996). Therefore, the present study aimed to investigate the effect of a synthetic GnRH (Fertagyl®) injection during summer on testosterone ,estradiol 17β and cortisol levels and lipidogram (Total lipids, triglycerides and cholesterol) in male farm animals.

MATERIALS AND METHODS

The present study was carried out at the National Research Center Experimental Farm, Abou RawashAbou Rawash, Giza, Egypt during summer (June-August,1999).

Six healthy mature cow bulls(3-4 years and 420-450 kg live body weight), 6 mature buffalo bulls (4-5 years and 450-540kg), 6 mature rams(2-3 years and 35-40 kg) and 6 mature bucks(2-3years and 30-35 kg) were randomly chosen for conducting the present experiment. Males were kept free away from females under open sheds with average ambient temperature of 33.33 Cβ ,relative humidity of 77.30%,and daylight of 13.67 hrs. Concentrated mixture (5 and 1kg /head/day for large and small males, respectively ; crude protein 15%), rice straw and water were provided *ad libitum*.

A synthetic GnRH, FertaglyÆ produced by the Intervet, the Netherlands in aqueous form. Each ml contains 0.1mg gonadorelin for single intramuscular injection to improve the fertility of farm animals. The recommended dose is 250μg (2.5 ml) and 100μg (1ml) for large and small animals.

respectively (Conn,1994).

Males of each species were divided into two equal comparable groups. The first group was kept as a control. Each male of the second group was injected (i.m) with the recommended dose of Fertagyl®, while the control group was injected with the corresponding volume of saline. Blood samples were obtained from the jugular vein of both groups into heparinized tubes before Fertagyl® injection (0 time) then at an hour interval for six consecutive times taking in consideration to expose animals to possible minimum stress during sampling. Samples were kept in ice box till centrifugation (X 3000g/ 15 minutes at 4°C). Plasma samples were harvested in aliquot vials and kept at 20°C till biochemical analysis.

Testosterone and estradiol 17β (Abraham,1981) and cortisol (Kowolaski and Paul,1976) levels were assayed by RIA using commercial kits from Diagnostic Product Corporation(Los Angles,USA). Assays had sensitivities of 0.04 ng/ml, 8pg/ml and 0.025 μg/dl with inter- and intra-assays c.v. of <13 &<13 ;5.65 & 5.30 and 5.17 & 4.70 for the above mentioned hormones, respectively. Total lipids (Frings and Dunn,1970), triglycerides (Wahlefeld, 1974) and cholesterol (Stein,1986) concentrations were colorimetrically analyzed using commercial chemical kits from Stanbio(Texas, USA).

Data were computed (SAS,1988) and statistically analyzed using one way analysis of variance , Student (t) test, regression analysis and correlation coefficients according to Snedecor and Cochran (1980).

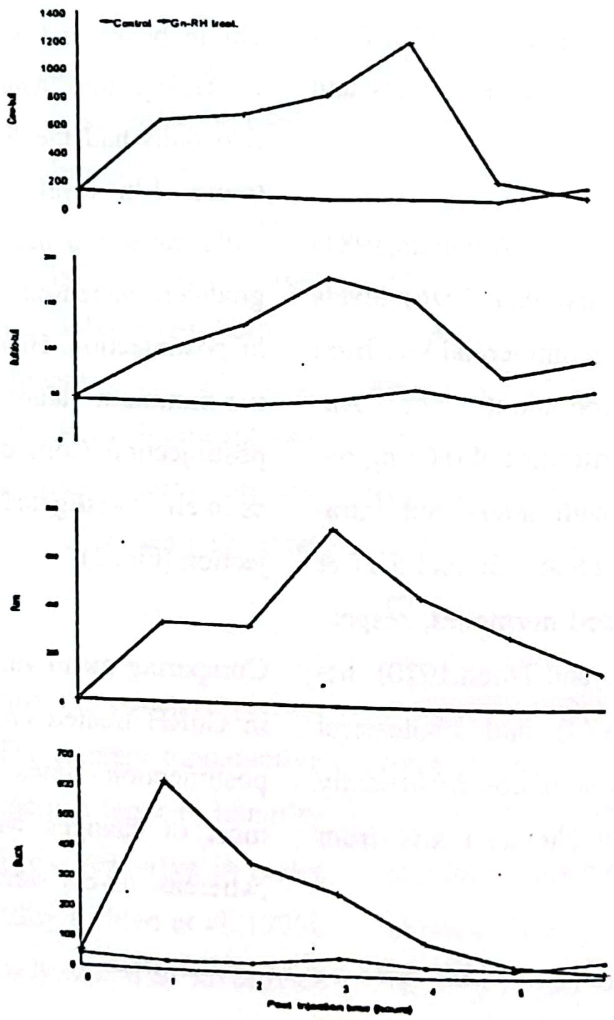
RESULTS

The effect of GnRH injection on mean plasma testosterone, estradiol 17β and cortisol profiles in cow-bulls, buffalo-bulls, rams and bucks is shown in tables (1-3) and figs. (1-3). Plasma testosterone level gradually increased in comparison with that of the control group with peak values obtained 1hr in bucks and 3-4 hrs in other species after GnRH injection. Among the studied species , buffalo bulls had the lowest testosterone level. Estradiol 17β level gradually decreased in cow bulls, rams and bucks, while in buffalo-bulls, it gradually increased with a peak value obtained 1 hr postinjection. However, in all studied species, the minimum values were recorded at the 6th hr postinjection. Cortisol levels revealed little changes in all investigated species following GnRH injection (Fig. 3).

Comparing mean values of the studied hormones in GnRH treated and control groups during the postinjection times (1-6 hours), revealed that most of changes were detected in testosterone ,whereas, levels were mostly higher ($P<0.01$) in all treated species(Table 1). Estradiol 17β level mostly decreased (P<0.01) in treated cow-bulls, rams and bucks, however, in buffalo-bulls, the level-increased (P<0.01) during the first 2 hrs postinjection, then decreased (P<0.01) during the 6th hr. postinjection in the treated group (Table 2). Cortisol level in all studied males revealed little changes during different hours post GnRH injection (Table 3).

Mean values of total lipid, triglycerids and cholesterol in male farm animals following GnRH injection were recorded in tables (4 -6). Analysis of variance indicated non significant variations in lipidogram values following GnRH injection. Regression analysis revealed non significant increases in total lipid and triglycerids, however, cholesterol values increased with the advance of time following GnRH injection in all studied species.

Figure (1) Effect of GnRH injection on during summer the level of Plasma testosterone (ng/dl) in farm animals.



In GnRH injected animals, testosterone levels significantly correlated with estradiol 17β in rams($r = -0.600, P<0.01$), while cortisol levels correlated with estradiol 17β in cow ($r = 0.801, P<0.01$) and buffalo($r = -0.495, P<0.05$) bulls as well as in rams($r = 0.528, P<0.05$).

Correlation coefficients between studied hormones and lipidogram values were reported in table (7). The most prominent correlations were found between testosterone and cortisol in one hand and total lipids on the other hand.

Figure (2) Effect of GnRH injection during summer on the level of estradiol-17β (pg/ml) in the plasma of farm animals.

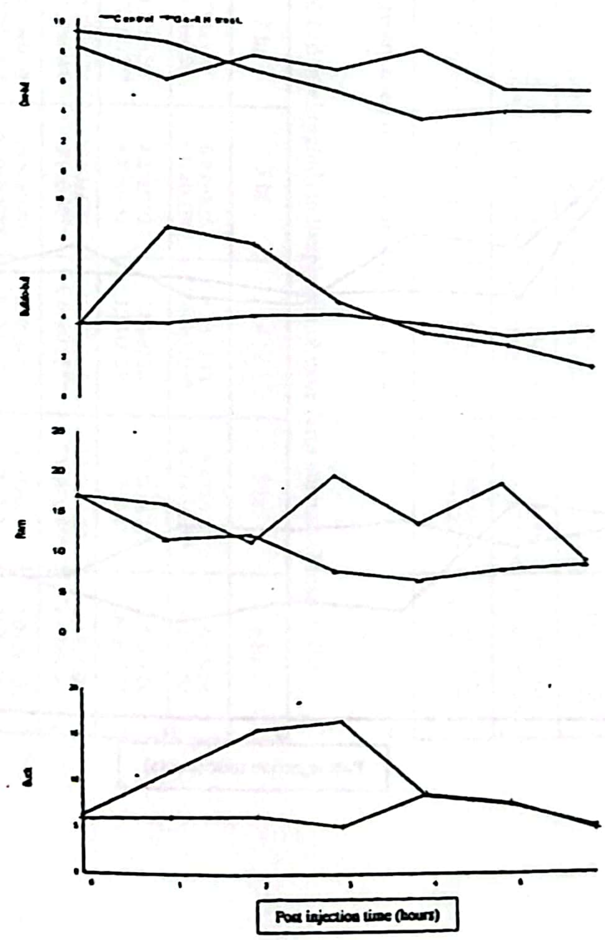


Figure (3) Effect of GnRH injection during summer on the level of cortisol (µg/dl) in the plasma of farm animals

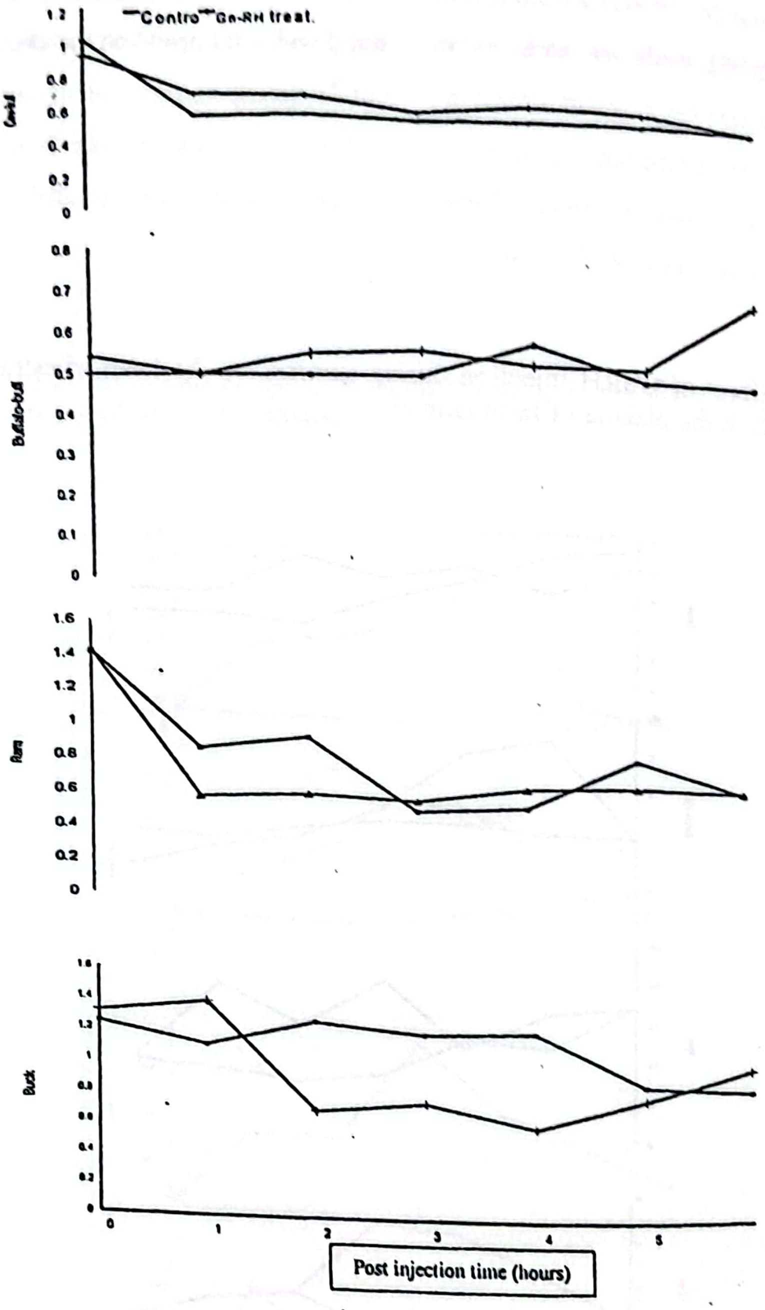


Table (1): Effect of GnRH injection during summer on testosterone level (ng/dl) in the plasma of male farm animals (Mean ± SE).

Animals	Group	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Cow-bulls	Control Treated	126.51±2.43 128.51±3.89	100.71±5.50 623.96±37.22**	86.96±1.76 665.03±27.14**	71.83±2.77 802.66±3.6**	78.36±2.42 1182.15±37.42**	78.50±2.56 208.13±3.69**	180.51±3.51 112.74±5.99**
Buffalo-bulls	Control Treated	45.46±1.63 48.50±3.56	44.66±0.98 97.86±2.17**	40.87±1.11 128.35±0.89**	47.98±2.40 180.46±4.19**	61.29±3.99 157.39±7.98**	46.36±2.44 76.01±7.47**	62.71±4.45 95.90±7.95*
Rams	Control Treated	17.71±0.87 19.74±0.85	16.33±1.48 342.09±16.31**	18.23±0.55 333.79±17.16**	19.03±1.48 743.83±68.87**	17.45±0.73 466.73±21.97**	17.64±0.88 312.10±17.16**	22.67±0.01 168.40±20.96**
Bucks	Control Treated	43.23±4.28 56.04±4.59	29.80±1.69 622.81±16.87**	31.56±2.37 361.05±26.04**	59.43±6.99 270.97±7.01**	34.03±2.22 115.03±10.87**	26.88±0.62 41.56±1.25**	52.53±3.53 15.76±1.47**

P<0.01 * P<0.5 **Number of animals is 3 in each group used for collection of 7 samples

Table (2): Effect of GnRH injection during summer on estradiol 17β level (pg/ml) in the plasma of male farm animals (Mean ± SE).

Animals	Group	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Cow-bulls	Control Treated	8.17±0.33 9.23±0.43	6.07±1.54 8.67±0.47	7.73±1.47 6.73±0.38	6.83±0.16 5.37±0.18**	8.23±0.43 3.60±0.05**	5.67±0.33 4.21±0.09**	3.67±0.24 4.27±0.52
Buffalo-bulls	Control Treated	3.70±0.16 3.67±0.09	3.77±0.55 8.63±0.46**	4.17±0.52 7.78±0.13**	4.27±0.93 4.87±0.32	3.80±0.20 3.33±0.21	3.23±0.21 2.73±0.21	3.51±0.28 1.68±0.01**
Rams	Control Treated	17.33±0.87 17.67±0.87	16.00±0.57 11.66±1.44**	11.33±0.87 12.27±2.44	19.67±0.66 7.83±0.60**	13.67±0.66 6.67±0.33**	18.74±0.27 7.97±0.55**	9.17±1.02 8.67±0.44
Bucks	Control Treated	6.27±0.23 5.84±0.50	11.06±0.52 6.10±0.55**	15.60±0.30 6.23±0.14**	16.50±0.40 5.10±0.45**	8.37±0.32 8.53±0.29	7.33±0.33 7.55±0.09	5.07±0.43 4.67±0.29

P<0.01 * P<0.5 **Number of animals is 3 in each group used for collection of 7 samples

Table (3): Effect of GnRH injection during summer on cortisol level (µg/dl) in the plasma of male farm animals (Mean± SE).

Animals	Group	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Cow-bulls	Control	1.01±0.05	0.55±0.05	0.56±0.03	0.52±0.02	0.53±0.03	0.54±0.04	0.55±0.05
	Treated	0.92±0.07	0.68±0.05	0.67±0.03*	0.57±0.03	0.62±0.08	0.61±0.06	0.55±0.03
Buffalo-bulls	Control	0.54±0.07	0.50±0.01	0.50±0.01	0.50±0.01	0.58±0.09	0.50±0.01	0.50±0.01
	Treated	0.50±0.01	0.50±0.01	0.55±0.09	0.56±0.09	0.53±0.06	0.53±0.05	0.70±0.23
Rams	Control	1.42±0.37	0.86±0.18	0.92±0.07	0.50±0.01	0.53±0.02	0.82±0.09	0.64±0.09
	Treated	1.42±0.13	0.57±0.04	0.59±0.09*	0.56±0.06	0.64±0.08	0.66±0.09	0.65±0.02
Bucks	Control	1.24±0.10	1.10±0.14	1.26±0.25	1.20±0.19	1.21±0.05	0.88±0.05	0.85±0.03
	Treated	1.32±0.26	1.37±0.14	0.69±0.19	0.75±0.13	0.60±0.06*	0.79±0.01	1.00±0.09

****P<0.01 * P<0.5 Number of animals is 3 in each group used for collection of 7 samples**

Table (4): Effect of GnRH injection during summer on concentration of total lipids (mg/dl) in the plasma of male farm animals (Mean ± SE).

Animals	Group	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Cow-bulls	Control	901.65±4.62	954.25±3.03	974.95±14.16	926.64±51.24	886.15±32.73	856.25±57.97	849.31±39.21
	Treated	844.05±35.35	892.23±42.84	912.62±45.78	1026.45±34.5	939.89±23.45	980.69±71.67	977.56±27.5
Buffalo-bulls	Control	510.96±34.29	447.05±53.46	543.85±54.39	517.97±50.19	536.97±32.59	518.67±36.98	495.41±36.72
	Treated	529.01±41.39	581.84±56.46	591.27±6.60	610.17±46.86	592.27±14.75	602.59±2.74	680.88±21.49
Rams	Control	774.78±34.8	852.80±24.29	730.01±11.72	869.23±89.57	734.61±128.5	740.72±67.38	668.06±20.11
	Treated	695.74±59.43	667.46±37.86	809.05±79.14	821.75±21.26	738.41±67.92	774.42±29.56	768.28±53.02
Bucks	Control	589.33±24.30	612.92±28.77	686.79±39.08	695.77±54.85	675.46±60.99	808.71±56.73	595.51±2.9
	Treated	624.4±31.24	654.7±41.66	775.72±8.59	636.37±55.99	643.53±57.37	617.76±72.68	615.59±58.01

****P<0.01 * P<0.5 Number of animals is 3 in each group used for collection of 7 samples**

Table (5): Effect of GnRH injection during summer on concentration of triglycerides (mg/dl) in the plasma of male farm animals (Mean ± SE).

Animals	Group	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Cow-bulls	Control	97.65±7.22	103.66±54.6	112.97±63.6	119.1±29.19	125.08±47.9	124.53±43.0	98.8±34.68
	Treated	126.03±24.5	147.0±48.39	146.07±15.3	134.23±32.1	135.43±21.4	133.43±7.81	141.53±46.1
Buffalo-bulls	Control	218.8±11.35	193.63±5.53	221.67±16.4	215.8±9.64	217.3±11.8	203.33±6.42	214.23±13.3
	Treated	202.57±9.69	221.13±12.8	230.23±19.5	220.47±13.1	221.5±25.49	221.5±25.49	213.03±47.4
Rams	Control	240.87±25.57	227.27±33.16	257.9±27.42	202.4±18.45	212.13±6.82	214.85±10.99	250.62±22.58
	Treated	231.51±22.01	255.74±20.48	283.37±19.81	227.4±17.89	227.63±26.65	249.77±7.22	208.73±14.54
Bucks	Control	245.23±5.85	278.03±20.88	244.87±13.98	240.4±20.05	244.77±12.49	244.26±38.39	241.14±24.23
	Treated	245.45±8.84	254.81±17.82	257.13±25.60	227.29±21.84	244.27±19.05	218.75±25.74	206.21±15.28

Number of animals is 3 in each group used for collection of 7 samples

Table (6): Effect of GnRH injection during summer on concentration of Cholesterol (mg/dl) in the plasma of male farm animals (Mean±SE).

Animals	Group	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Cow-bulls	Control	197.13±1.16	193.4±12.06	201.3±14.01	209.37±9.85	189.8±6.34	185.03±16.32	183.10±16.29
	Treated	178.43±20.01	230.37±6.99	222.13±7.68	173.0±20.24	174.63±17.50	191.1±10.74	180.3±6.93
Buffalo-bulls	Control	163.43±4.13	161.2±9.08	154.56±1.7	154.47±13.55	143.17±14.71	140.7±9.63	144.77±10.08
	Treated	156.36±15.97	190.13±8.33	159.67±10.37	169.61±15.57	169.97±18.70	181.53±13.89	184.37±5.37
Rams	Control	168.3±9.34	172.7±13.75	176.33±10.37	179.6±3.77	160.27±6.01	169.43±17.33	148.73±10.9
	Treated	154.56±12.11	168.1±16.95	150.86±14.72	160.43±7.61	180.0±12.68	143.2±6.91	194.95±12.79
Bucks	Control	187.67±18.18	177.27±1.46	187.27±25.47	199.91±8.61	188.86±13.1	151.97±18.45	152.75±23.26
	Treated	148.9±8.66	183.91±8.74	158.33±10.09	156.4±4.51	141.61±5.75	145.86±5.15	151.03±14.64

Number of animals is 3 in each group used for collection of 7 samples

Table (7): Correlation coefficients among hormone levels and lipidogram in the plasma of male farm animals after GnRH injection during summer.

Animals	Hormones	Total lipids		Triglycerides		Cholesterol	
		Control	Treated	Control	Treated	Control	Treated
Cow-bulls	Testosterone	-0.391	0.443*	- 0.819**	- 0.544**	- 0.456*	0.031
	Estradiol-17	0.489*	- 0.561**	0.286	0.012	0.551**	0.479*
	Cortisol	-0.047	- 0.623**	- 0.548**	0.277	0.118	0.072
Buffalo-bulls	Testosterone	0.057	0.287	0.178	0.590**	- 0.606**	- 0.075
	Estradiol-17	0.250	-0.374	0.443*	0.514*	0.474*	- 0.021
	Cortisol	0.359	0.902**	0.362	0.078	- 0.129	0.299
Rams	Testosterone	- 0.550**	0.520*	0.362	0.028	- 0.625**	- 0.042
	Estradiol-17	0.681**	- 0.427*	0.600**	0.471*	0.582**	- 0.352
	Cortisol	0.004	-0.437*	0.431*	- 0.239	0.120	- 0.189
Bucks	Testosterone	- 0.369	0.491*	- 0.459*	0.659**	0.249	0.912**
	Estradiol-17	0.235	0.044	0.068	0.298	0.658**	0.431*
	Cortisol	- 0.221	- 0.301	0.044	0.173	0.939**	0.496*

** P < 0.01
* P < 0.05

DISCUSSION

Reproduction in mammals is under the control of the hypothalamus, the pituitary gland and gonads. This control is mediated by the gonadotrophic releasing hormones GnRH which is produced and secreted by the hypothalamic neurons. Moreover, GnRH provide a humoral link between the neural and endocrine systems (Conn, 1994).

It appeared from this study that testosterone was the most affected hormone following GnRH injection as levels significantly increased in the plasma of treated males and attained peak values 1-4 hrs postinjection according to species. These results are in agreement with the results obtained in buffalo bulls (Abdel-Malak et al., 1992; Mehta et al., 1992), cow-bulls (Thompson et al., 1992; Kozdera et al., 1993) and rams (Xu et al., 1991).

GnRH act directly on the pituitary gonadotropins to release LH in a dose related manner (Conn, 1994). LH response is much greater and more prolonged than FSH (Padmanabhan et al., 1995). Synthetic GnRH was found to be qualitatively the same as the natural GnRH decapeptide but quantitatively 50-70 times more potent in LH release stimulating and have the ability not only to stimulate the release of LH, but also to sensitize the pituitary gland as well (Conn, 1994).

It is evident that GnRH combine and activate specific plasma membrane receptors which vary dynamically with the changing endocrine milieu of the animal. Some of the changes in GnRH receptor numbers is mediated at least in part via changes in GnRH-receptor gene transcription (Padmanabhan et al., 1995). GnRH receptors complex routed to two distinct intracellular compartments; the lysosomes and Golgi apparatus (Badr and Pelletier, 1989) for releasing of gonadotropin via Ca++ dependent mechanism involving receptor-mediated phosphoenositide hydrolysis (Conn,1994). Moreover, the condition had been confirmed in vitro whereas, GnRH stimulated gonadotrophin polypeptide biosynthesis (Starzec et al., 1986) and glycosylation (Vogel et al., 1986) in cell culture.

Estradiol 17? levels decreased after GnRH injection in all studied species except in buffalo, whereas the level increased mostly during the first 2 hrs, then decreased significantly during the 6th hr postinjection . In this respect, it was reported that estradiol 17? profoundly influences GnRH secretion in sheep and it not only regulates the frequency and amplitude of GnRH pulses, but also produces quantitative changes in its pattern of release (Evans et al., 1995 ; Hamernik et al., 1995).

Species Variation n pulses, but also produces quntitative changes in its pattern of release amernik et al.,1995).The in the pattern of testosterone and estradiol 17? in response to GnRH may be due to these steroids are secreted by separate compartments in the testis (Schanbacher and Echternkamp, 1978; Stein,1986). Moreover, species difference in synthesis and conversion of steroids could be expected, as it is well established that in steroid biosynthesis ,estradiol is cleaved from testosterone (Stryer, 1996). The low testosterone level in buffalo-bulls is in agreement with the findings of Ahmed and Nada (1994) who attributed the low sexual desire in this species to the insufficient thermoregulatory mechanism.

In the present study, application of GnRH in male farm animals induced little stressful reaction as monitored by cortisol level. In this respect , it was previously recorded that GnRH induced no significant changes in kidney and liver functions or blood cytology of goats(Mohsen,1993).

The little increases in total lipids and triglycerides values post GnRH injection may be due to the increased LH secretion (Rasby et al., 1992), while the little decreases of cholesterol may be related to steroidogenesis (Stryer, 1996).

The present study showed marked negative correlation between testosterone and estradiol 17? levels in rams. Such correlation was previously recorded by Hamerik et al.(1995) who added that estradiol increased the amount of mRNA for GnRH receptors and regulate LH secretion . The positive correlations between estradiol 17? and cortisol in bulls and rams herein may be due to increased ACTH secretion following GnRH injection (Forbes et al., 1994). The reported correlations between studied steroid hormones and lipidogram were in line with the finding of Cunnigham (1977) who reported that all aspects of lipid metabolism affect steroidogenesis.

In conclusion, injection of GnRH during summer in male farm animals (whereas reproductive performance is expected to be low) improved sexual desire as manifested by high testosterone levels. However, studies on semen attributes are being prepared for publication in a separate paper. It is recommended that GnRH application should be used in high fertile animals exhibiting unsatisfactory libido especially during summer season.

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